2333-Pos Board B470

Stochastic Control of Intracellular Latency and Transactivation in Human Immunodeficiency Virus Type 1 (HIV-1)

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Efforts for curing HIV infection have been largely unsuccessful. A major obstacle is that the virus forms latent reservoirs in resting CD4+ T cells, which cannot be eradicated by current antiretroviral therapies. However, latently infected cells can be activated at later times to enter the transactivation state of viral production. The selection between latency and transactivation are likely stochastically controlled. However, detailed control mechanisms are unknown. To address this issue, we have developed a stochastic model for the intracellular genetic circuit controlling HIV latency and transactivation, and computed directly the steady state and time evolving probability landscape using the multi-finite buffer discrete Chemical Master Equation (mb-dCME) method. The computed stochastic steady state landscape of the HIV Tat circuit exhibits bistable behavior, with a highly stable latent state with zero Tat protein and a much wider but less stable transactivation state of nonzero Tat protein. Our calculation results agree with experimental observations of latency and transactivation dynamics. To further examine how the rate of intracellular HIV-1 transactivation is affected by the positive feedback of Tat circuit, we construct a mono-stable circuit by removing this feedback. We show that the transactivation rate in the mono-stable circuit is significantly lower than the wild-type bistable circuit. To study how the HIV-1 provirus can be transactivated more effectively, we systematically explored different biochemical properties of Tat binding, reaction rates, and transportation rates between nucleus and cytoplasm. Our results suggest novel approaches for eradicating HIV-1. Our results show that the simple positive feedback loop of Tat self-activation is sufficient to generate bistability, without the need for cooperativity and negative feedbacks. Our approach is general and can be applied to study broad issues in genetic circuits modeling in systems biology.

2334-Pos Board B471

Path Attractors and the Origins of Stochastic Bifrucation and Dephasing in Genetic Networks

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Gene regulatory networks are driven stochastic systems with the noise having two distinct components due to the to birth and death of metabolite molecules and dichotomous nature of gene state switching. Presence of dichotomous gene noise alone has the capacity to significantly perturb the optimal transition paths and steady state probability distributions compared to the macroscopic models and their weak noise approximations. Most importantly dichotomous gene noise can also lead to multimodal distributions due to stochastic bifurcation of the underlying nonlinear dynamical system, which underlies the mechanism of formation of population heterogeneity. In this note we derive approximate path based expression of the time dependent probability of gene circuits which enables deeper exploration of the role of gene noise in formation of epigenetic states and dephasing-like phenomena.

2335-Pos Board B472

Worms in Space: Epigenetic Response of C. Elegans in Simulated Microgravity

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Biological evolution is directed by the environment, and life on our planet is adapted to, and is optimized for growth and reproduction in Earth's gravitational field. The effects of altered gravity on biology, however, is not well understood, especially microgravity because of the limited access to space. As humans begin a new era of exploration in low Earth orbit and beyond, it will be important to understand how biological processes respond to such drastic change in the gravitational environment, and if any long term (*i.e.,* multi-generational) consequences exist. We present a new tool for studying biophysics in microgravity, "ZOOM" (Zero-gravity On-Orbit Microscope). ZOOM is a free-flying (3U) nanosatellite with a scientific instrument payload that enables diffraction-limited bioimaging in space. A ground-based ZOOM simulator, "ZOOM-Sim", utilizes the same microscope mounted onto a clinorotation apparatus. We demonstrate that ZOOM-Sim is able to mimic microgravity conditions by mapping the trajectories of microspheres suspended in solution, and compare experimental results with theory. For biophysical experiments, we use ZOOM-Sim to monitor the development of *C. elegans* in simulated microgravity, followed by high-throughput epigenetic sequencing to elucidate the genetic responses due to gravity unloading. We compare the epigenetic profiles of five histone marks (H3K27me3, H3K4me3, H3K9me3, H4K20me1, H3K36me1) and RNA Polymerase II binding sites from animals exposed to simulated microgravity with 1×g controls, and identify putative gravity-responsive genes in *C. elegans* and homologs in humans. These studies will increase our understanding of how gravity (and lack of) influences biological processes, in preparation for future human space exploration.

2336-Pos Board B473

Stochastic Multistability from Simple Network Motifs

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Studying stochasticity in gene regulatory networks is essential for understanding important cellular processes, such as those involved in determining cellular fate. However, studying stochasticity is a challenging task. For example, stochastic simulation algorithm is inefficient in examining rare events. Direct solution of the discrete Chemical Master Equation requires enumeration and truncation of usually enormous state space. We developed the Multi-Finite Buffer method for direct solution of discrete Chemical Master Equation (mb-dCME method), which allows the probability landscape of a large class of stochastic networks to be computed exactly. Here we use the mb-dCME method to study the probability landscape of simple network motifs. Our results show that complex behavior such as multistability can arise in very simple motifs without feedback loops and cooperativity.

Synthetic Biology

2337-Pos Board B474

Metabolic Channeling and Spatial Effects of Bifunctional Enzymes

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Synthetic multi-enzymes complexes (MECs) hold promise to increase metabolic fluxes and reduce undesirable and hard-to-characterize contextual interactions with host systems. A major advantage of MECs in metabolism is referred as metabolic channeling [1] wherein a product is channeled to the subsequent active site in the MECs. Yet this mechanism is not very well characterized. We develop a compartmentalized minimal model of two sequential metabolic reactions. We assume that a "vicinity volume" surrounds each enzyme, where the corresponding binding reaction happens. Reactants are assumed to be well-mixed inside the vicinity and in the bulk solution. We explore the advantages of metabolic channeling by comparing bifunctional enzymes (BIFE) and its two corresponding monofunctional enzymes (MOFE) side-by-side using both theoretical analysis and simulations. The theoretical analyses are based on classic Michaelis-Menton Kinetics, the diffusion-controlled reaction rate and the diffusive reaction rate formulated in [2]. Smoldyn is used for simulations [3]. We find that the level of substrate channeling is determined by a governing dimensionless group α, which is the ratio between the diffusive timescale and the reactive timescale. The steady state level of the reactant in the bulk solution in BIFE system is always lower than the MOFE system and α determines the level of this reduction. The dynamical properties also show important differences. Reaction flux is increased in a short timescale in BIFE system compared to MOFE system, and the level of the increase is again determined by the dimensionless parameter α. This increase in flux vanishes after both systems reach the steady state.

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2338-Pos Board B475

Surface Display of a Unique Enzyme as a New Reporter in Synthetic Biology

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Synthetic biology is a relatively new area of engineering, with engineered gene circuits like bistable switches and oscillators being introduced about fifteen years ago. Biophysics plays a role in synthetic biology both in the analysis of the dynamics of these synthetic gene networks, as well as the synthesis of novel structural components. Here, we describe an approach to create a new structural component that also allows the investigation of synthetic gene circuit dynamics. This component allows for reporter activity such as that enabled by fluorescent proteins, while also allowing biophysical and biomaterial interactions with the cellular environment. Specifically, we have genetically engineered surface display of SNAP. The SNAP protein can bind to any benzyl guanine (BG)-conjugated molecule such as synthetic fluorescent dyes or other molecules with detectable properties (e.g., magnetic beads, which could be used in potential cell sorting approaches). SNAP is derived from O6-alkylguanine-DNA-alkyltransferase. We displayed it on the surface of E. coli cells by linking it to Lpp-OmpA, a protein created by fusing the first nine N-terminal amino acids of the lipoprotein (Lpp) to amino acids 46-159 of outer membrane protein A (OmpA), allowing fluorescent and magnetic particles to bind to the cell surface and serve as a reporter of synthetic gene network behavior. SNAP display on the surface of cells can be confirmed through microscopic observation and flow cytometry. We anticipate this new reporter will have broad impact fields ranging from cell wall and membrane biophysics to synthetic biology and material science.

2339-Pos Board B476

A Multiscale Dissection of Decision-Making in Microbial Ecosystems

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Microbial ecosystems in nature are typically composed of hundreds or thousands of species, heterogeneously distributed in space and time. Interactions between these microorganisms help regulate the overall activity and functional outputs of these ecosystems. We have been applying the principles of quantitative biology to understand regulation in multispecies communities of microbes. By approaching ecosystem regulation at multiple length scales, ranging from transcriptional decisions made at the molecular level to the propagation of functional states through signal exchange, we aim to develop a predictive understanding of the collective properties of these diverse cellular networks.

Towards this goal, I will discuss our efforts to predict global regulatory outputs of individual cells in response to changes in environmental conditions, using iron-mediated regulation in Pseudomonas aeruginosa as a specific example. Regulatory decisions are modulated by multiple factors, and through a combination of theoretical approaches and the tools of synthetic biology to tune regulatory parameters inside of cells, we attempt to uncover how information from multiple inputs is integrated at the promoter level.

Given the diversity of most microbial community in the real world, we then examined how such regulatory decisions at the single-cell level are shaped by local interactions between different species. At the ecosystem level, I will present theoretical and experimental results examining the ability of changes in regulatory states to be communicated via diffusible signals. We explored how ecosystem composition, the spatial distribution of cells, and the mechanistic details of communication pathways influenced the potential for global coordination of activity within the ecosystem.

2340-Pos Board B477

Engineering Electron Transferring Proteins and their Assembly at Electronic Interfaces

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Many key cell functions are accomplished through complicated system of enzymes and redox carrier molecules that control electron and proton transport. Although significant number of these enzymes has been structurally characterized, the actual mechanism of redox catalysis is not always understood. Therefore we have adopted a different approach to address the structure-function relationship of oxidoreductases: we aim to uncover the assembly instructions required for function using smaller, simpler, more robust model proteins, maquettes. Our questions ask how many engineering elements are required to achieve a particular biological function, what are the individual biochemical and structural tolerances of these elements and how much of a protein infrastructure is consumed in accommodating the function. To start answering these questions, we have synthesized a set of amphiphilic maquettes. These maquettes transfer electrons across membranes, bind O2 and CO. They can be assembled on electrodic substrates through a variety of attachment strategies including simple adsorption, cysteine attachment to gold, histidine attachment to Ni-NTA, and click chemistry.

2341-Pos Board B478

Automated Design of Enzyme-Driven DNA Circuits

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Molecular programming allows for the bottom-up engineering of biochemical reaction networks in a controlled in vitro setting. These engineered biochemical reaction networks yield important insight in the design principles of biological systems and can potentially enrich molecular diagnostic systems. The DNA-based polymerase-nickase-exonuclease (PEN) toolbox has recently been used to program oscillatory and bistable biochemical networks using a minimal number of components. Previous work has reported the automatic construction of in silico descriptions of biochemical networks derived from the PEN toolbox, paving the way for generating networks of arbitrary size and complexity in vitro. Here, we report an automated approach that further bridges the gap between an in silico description and in vitro realization. A biochemical network of arbitrary complexity can be globally screened for parameter values that display the desired function and combining this approach with robustness analysis further increases the chance of successful in vitro implementation. Moreover, we present an automated design procedure for generating optimal DNA sequences, exhibiting key characteristics deduced from the in silico analysis. Our in silico method has been tested on a previously reported network, the Oligator, and has also been applied to the design of a reaction network capable of displaying adaptation in one of its components. Finally, we experimentally characterize unproductive sequestration of the exonuclease to phosphorothioate protected ssDNA strands. The strong non-linearities in the degradation of active components caused by this unintended cross-coupling are shown computationally to have a positive effect on adaptation quality.

2342-Pos Board B479

Coupling the Increase in Membrane Tension and the Synthesis of Phosphatidylserine in a "Smart" Artificial Cell

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Assembling biological parts into a functional system using a bottom-up in vitro reconstitution approach offers the possibility of designing artificial cells with the ability of sensing and responding to external stimuli. An artificial platelet, which is programmed to mimic the functionality of a natural platelet, is our first test-bed for this design strategy. The greatest challenge of building the artificial platelet is the coupling of external mechanical stimuli to a biochemical response. We have reconstituted mechanosensitive channels (MscL) and phosphatylserine synthase (PSS1) into a liposome using cell free expression so that the liposome can sense an increase in membrane tension and allow influx of calcium ions, which serve as the second messengers to activate the synthesis of phosphatidylserine (PS). We used double emulsion template to generate lipid bilayer vesicles with encapsulation capability. We have also developed an in-house cell free expression technique for expressing MscL and PSS1. By encapsulating the cell free expression components inside the lipid vesicles, we have shown that the membrane proteins successfully insert into the lipid bilayer membrane and function properly. We also showed that the system can couple the increase in membrane tension and the synthesis of PS. The success of this work provides a great leap forward in achieving our ultimate goal, which is to build an artificial platelet.

2343-Pos Board B480

Light-Controlled Growth Factor-Mediated Signal Transduction

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Growth factor-mediated signal transduction regulates critical cellular responses including cell proliferation, differentiation, migration, and apoptosis. However, direct and quantitative linkage between time kinetics of growth factor-mediated pathways and the cellular response is still lacking due to difficulties in perturbing the kinetics of intracellular signaling pathways. Here, we construct an optogenetic system that uses light to regulate growth-factor mediated signaling pathways. We show that various downstream pathways control cell fate determination differentially. Overall, light-controlled growth-factor signal transduction enables precise dissection of individual subsets of signaling pathways in cells.

2344-Pos Board B481

Patterned Biofilms for Engineering Microbial Consortia

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Over the past decade, synthetic biology has developed increasingly robust gene networks within single bacterial cells, but relatively few systems have demonstrated engineered multicellular behaviour. In contrast, naturally existing terrestrial bacteria primarily live in complex surface-attached communities known as biofilms. Within these biofilms, multiple distinct microbial sub-populations form intricate spatial structures including colony co-localization and cell-cell co-aggregation. This spatial organization allows bacterial communities to achieve cooperative behaviours such as metabolic division-of-labour, and conversely, ecological interactions between different microbial subpopulations in turn influence the spatial patterning within the biofilm. Using optogenetic, metabolic, and cell-adhesion tools from synthetic biology, we are developing a biofilm culture platform that can generate optically patterned, metabolically interacting microbial communities. The platform will provide spatiotemporal control of both cell-surface and cell-cell attachment, tunable for both strength and specificity, as well as adjustable regulation of intercellular metabolic interactions. Taken together, these represent new tools to investigate how spatiotemporal patterning develops in biofilms and to engineer synthetic microbial consortia capable of complex tasks requiring biological division of labour.